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The Potential Application of Hairless Guinea Pigs as a Replacement for the Yucatan Mini-pig in Animal Studies

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The Yucatan mini-pig (*Sus scrofa*) is one of the most widely used animal models for skin damage studies because it shares many of the same physical properties as human skin. While the Yucatan is ideal for laser exposure studies using a large spot size, its size and cost are excessive for projects using smaller beams. This experiment performed histological analysis of skin biopsies from pigmented Hairless Guinea Pigs (*Cavia porcellus*) for epidermal thickness and melanin distribution. That data was then compared to similar information on the Yucatan.
Keywords: skin comparison, guinea pig, pigmentation, histology

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ABSTRACT

The Yucatan mini-pig (*Sus scrofa*) is one of the most widely used animal models for skin damage studies because it shares many of the same physical properties as human skin. While the Yucatan is ideal for laser exposure studies using a large spot size, its size and cost are excessive for projects using smaller beams. This experiment performed histological analysis of skin biopsies from pigmented Hairless Guinea Pigs (*Cavia porcellus*) for epidermal thickness and melanin distribution. That data was then compared to similar information on the Yucatan.

Keywords: skin comparison, guinea pig, pigmentation, histology

1. BACKGROUND

Safety standards for laser exposures are based on both computer models and experimental data obtained from animal studies. Because of the anatomical similarity to human skin[1], the most common animal used for skin damage assessments is the Yucatan mini-pig (*Sus scrofa*). They are ideal for large diameter spot sizes since they possess a lot of surface area along the flanks. However, Yucatans (Figure 1a) can be difficult to transport and house due to the amount of space required. For experiments using small spot sizes, a smaller animal model would be more economical for investigators.

Previous studies[2] have shown albino Hairless Guinea Pigs (*Cavia porcellus*) to have skin damage thresholds close to those of humans at 1540 nm. (Figure 1b) This animal proved to be easier to handle, more convenient to house, and less costly than the mini-pig (Mini-pig = ~$400 versus the pHGP at $150). Published research shows that hairless guinea pig skin performs much like human skin when it comes to normal biological processes like wound healing and toxicology[3,4,5]. Many of the papers stated that HGPs have similar skin properties as humans, but do not give any specific detail. Sueki et al[3] did a histological study of guinea pig skin but reported only qualitative results, not actual data values. Most of the research papers focused on nerve and toxicology studies with the predominant theme of the papers involving the effects of sulfur-mustard gas. Since most hairless guinea pigs are albino, we were unable to find any data on melanin content in the papers. Melanin is a critical factor when determining tissue absorption properties for lasers and precise measurements of its concentration, as well as the distribution, are crucial to predicting damage effects from directed energy. There is now a pigmented version of the hairless guinea pig (pHGP) available. It is hypothesized that if the melanin content is close to the levels seen in humans, then pigmented hairless guinea pigs (Figure 1c) should be suitable for experiments in the visible wavelengths. This study is designed to measure melanin distribution in the epidermis, as well as to determine thickness values for the epidermis and dermis. The results will be used to determine if this animal model is suitable for biophotonic skin damage threshold experiments.

Pigmented hairless guinea pigs come in two varieties: the Skinny and the Baldwin. The Skinny is the result of breeding between an albino HGP (genetically engineered by Canadian scientists for dermatological studies) and the normal haired variety. While not completely hairless, the animal does have significantly less hair than a regular guinea pig. What little hair they do possess is very thin and wispy. Some vendors inbreed the animals to ensure a large supply of the Skinny, but this

makes them susceptible to problems with their immune system. To avoid this, reputable breeders often breed their hairless Skinny with haired guineas for several generations. Since the offspring are combination litters of hairless and haired animals, this practice greatly reduces the number of animals available.

The Baldwin is genetically different than the Skinny. The mutation for Skinnys reduces the production ability of the hair follicles from birth, but Baldwins are actually born fully haired. At five days of age the hair begins to fall out and by the time it is six weeks old, the animal is completely hairless. The Baldwin is more fragile when it comes to health, making it difficult to breed. The pHGPs are typically 500-600 grams when fully grown and are very social. They prefer to be housed together, making transportation and housing fairly simple. .

Figure 1: (a) Yucatan Mini-pig **(b) Albino Hairless Guinea Pig** **(c) Pigmented Hairless Guinea Pig**

Due to the fact that there are very few breeders of pHGPs these animals are only available as they are born, and the litters are unpredictable in that the offspring can be either homozygous for the traits, or carriers of the mutation. Carriers do not show the hairless traits; instead they appear as normal haired guinea pigs. Because of the difficulties in breeding these guinea pigs, both types of animals are hard to obtain. Three separate USDA licensed vendors were approached for this experiment and all three said they had stopped breeding them. The lone supplier that was able to supply the animal used here stopped selling the animals within days of shipping the one used in this study, thus we were unable to obtain more than one animal.

As mentioned earlier, pHGPs are very fragile when it comes to their health. This study will monitor the animal post-operation for 48 hours. These observations will be used to determine the feasibility of using this animal based on its ability to cope with the stress of handling and surgical procedures.

As part of this study, we removed samples of blood from the animal in order to provide a genetic and chemical understanding of the guinea pig, as well as serving as baseline information for future studies. This was done at the same time of the biopsy in order to minimize stress on the animal.

2. METHODS

2.1 Animals

The animal involved in this study was procured, maintained, and used in accordance with the Federal Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources --National Research Council. Brooks City-Base, TX has been fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967.

One 10-week old male Skinny guinea pig (S&S Exotics in Houston, Texas) weighing 600 grams was studied[♦]. As noted earlier, the supplier stopped selling these animals after this first animal was purchased, thus he was the only subject available for testing. An anesthesia mixture of Domitor (0.5 mg/kg) and Telazol (40 mg/kg) was administered through intramuscular injection upon delivery to the surgical room. An analgesic injection of Meloxicam (1 mg/kg) was also administered at this time. The guinea pig was fed a standard guinea pig diet and given treats of cranberries and orange juice. Food was withheld for 12 hours prior to surgery, water was always available. Even though the animal had little hair, there was enough of it to interfere with data collection. Thus, he was carefully shaved using electric clippers and then gently cleansed with surgical scrub and warm water.

To assess the second goal of evaluating the animal's ability to withstand these procedures, the animal was placed back into group housing to recover. During this recuperation time, he was closely monitored for any adverse effects due to the biopsy process. Personnel observed the animal's activity level, appetite, and condition of the biopsy sites.

2.2 Sample collection

Each flank was inspected for any discoloration, scars, or other damage. Once suitable areas had been established, four biopsy sites were chosen on each flank. A 5-mm biopsy punch was used to cut out the sections of tissue. Each sample was placed in a 10% formalin solution, fixed in paraffin wax, cut in half, and then stained with Steiner Stain.

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* The animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act, "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources National Research Council, and DoD Regulation 40-33 Secnavinst 3900.38C AFMAN 40-401(1) DARPAINST 18 USUHSINST 3203 "The Care and Use of Laboratory animals in DOD Programs." Brooks City-Base, TX has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967.

BIOPSY:

1. Investigators evaluated the skin and chose locations for the skin excision.
2. A 5-mm biopsy punch was pressed against the skin and slowly rotated until cut through the designated section.
3. A surgical blade was used to slice the skin from the underlying tissue.
4. The biopsy sample was removed using forceps.
5. The sample was placed into a pre-labeled holder and then inserted into a jar of 10% neutral buffered formalin.
6. The biopsy site was blotted with gauze to minimize pooling of blood.
7. Investigators then proceeded to the next biopsy location.

CLOSURE:

1. Once all of the skin biopsies had been removed from a flank, investigators used tissue glue (Dermabond) to close those sites.

BLOOD: Biological handling and preparation of the blood samples was performed by qualified lab personnel. Due to the small size and fragility of these animals, only 1.0 mL of blood was drawn. Samples were run through a BCA Assay, and the results were plotted in Excel using a two-order polynomial curve fit.

BLOOD DRAW:

1. The blood draw was performed while the animal is under anesthesia.
2. Approximately 1.0 mL of blood was collected into room temperature Sarstedt Multivette 600 vacutainers. Lithium heparin is already contained in each of the vacutainers as an anticoagulant.
3. Blood samples were immediately placed into a container of dry ice for transport to the lab.

2.3 Sample Analysis

Epidermal thickness was measured at 400x magnification using an Olympus BX-50 microscope. Ten measurements, with intervals of 0.5-mm, were taken at sites randomly chosen along the sample. An intra-ocular micrometer within an Olympus WHN10X-H/22 eyepiece was used for the analysis. The epidermis was measured from the dermal-epidermal border to the place where the stratum corneum began to separate.

For determination of melanin content, the samples were observed at 1000x magnification, using a 10x10 grid reticule from Olympus. (Figure 1) The grid was placed over three sections of each sample, ensuring that all of the epidermis was included. This allows for viewing a $0.01mm^2$ area of skin. At each location, the visible epidermal cells were counted and the fraction of those cells that contained melanin was determined. Total skin melanin distribution was then calculated from these numbers.

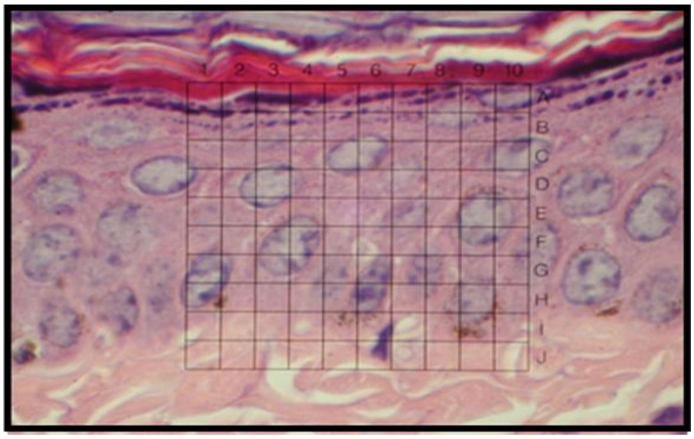

Figure 2: Overlay of the grid used for determining melanin distribution in the skin.

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BLOOD ANALYSIS:

1. **T**he collected blood samples were thoroughly mixed.
2. The containers were then placed on ice, and centrifuged at low speed and 4^oC until the red and white blood cells have separated from the plasma.
3. Plasma supernatant was removed by pipettor, leaving several millimeters of plasma above the cellular interface to avoid carryover. This supernatant was placed in a conical centrifuge tube on ice.
4. The plasma was then mixed again to ensure uniformity, and aliquots of 50 and 100 µL were placed into Eppendorf tubes and then snap frozen in liquid nitrogen.
5. 2D-protein gene analysis was performed using 100 µL of the pure plasma and a Bio-Rad precipitation kit.

3. RESULTS

A total of four samples were obtained from this animal. The animal seemed to tolerate the procedures well, alleviating concerns that the pHGP's fragile nature could lead to problems from the surgery.

The data shows less skin thickness variability for the pigmented hairless guinea pig than the Yucatan and human values reported by Eggleston[1]. (Table 1) Values for pHGP epidermal thickness ranged from 23µm to 49µm over both flanks regardless of location, while the Yucatan varied from 34µm to 92µm. (Table 2)

Mean thickness[a] of human[b] epidermis

Arm			Neck			Face		
Age (yrs)	Sex	Mean thickness (µm) ± SD	Age (yrs)	Sex	Mean thickness (µm) ± SD	Age (yrs)	Sex	Mean thickness (µm) ± SD
30	F	52 ± 11	57	M	78 ± 14	43	M	73 ± 21
36	F	82 ± 27	61	M	62 ± 7	51	M	51 ± 31
40	F	60 ± 17	70	M	58 ± 25	58	M	85 ± 34
65	M	74 ± 13	73	M	57 ± 26	60	F	72 ± 17
69	M	73 ± 25	73	F	63 ± 29	70	M	66 ± 22
76	M	61 ± 8	78	M	72 ± 27	76	M	57 ± 13
Mean		68 ± 21	Mean		65 ± 24	Mean		68 ± 26

[a]Measured from the junction of dermis and stratum basale to the separation of stratum corneum.
[b]Caucasian

Table 1: Human skin thickness data, taken from Eggleston et al., 2000

Skin Morphology		
	Epidermal Thickness (µm)	Melanin Distribution
Yucatan Mini-pig	68 (±34)	45-50%
pHGP	36.75 (±13)	25-30%
Human	68 (±21)	16-26%

Table 2: Summary of values. Yucatan and human values taken from previous studies[1].

The melanin distribution results show the pigmented guinea pigs to be much closer to the human values than the Yucatan. The Yucatan has almost triple the melanin distribution of human skin, while this animal showed similar distributions for the lighter pigmented areas. Even the darkest guinea pig skin tested had less melanin than the Yucatan's lightest area.

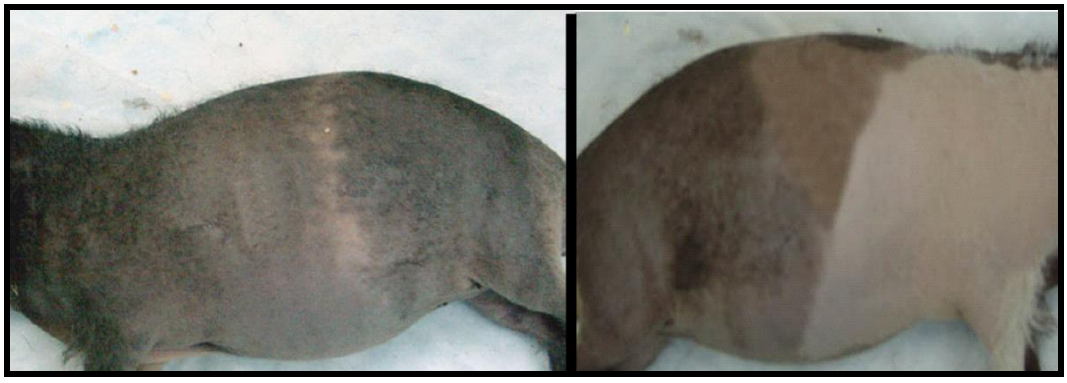

Figure 3: Pigmentation seen in test subject. Left flank and right flank.

Approximately 40 µL of blood plasma was collected, with the protein concentration determined to be 35.02 - 41.3 µg/mL.

4. CONCLUSIONS

As with the Yucatan, melanin content of the guinea pig skin can be extremely varied. (Figure 3) The melanin distribution was a lot closer to human skin than the Yucatan was, illustrating potential for this animal to be used in laser skin damage studies.

One issue that complicated an accurate comparison of this data was the different stains used for histological analysis. The study performed by Eggleston used hematoxylin and eosin stain (H&E), which is not a melanin-specific stain. With H&E stain, melanin shows up as purple blotches over a purple tissue, making many of the granules difficult to see. (Figure: 4b) The stain used for this guinea pig study, however, was melanin-specific. Melanin appeared as black particles over a tan background, making them stand out from the surrounding tissue. (Figure: 4a) Upon comparison of two slides stained with each of the respective stains, it was clear that the Steiner stain was much more efficient at making the melanin visible for counting. Unfortunately, no swine samples were available to be stained with the Steiner stain, so a direct comparison was not possible.

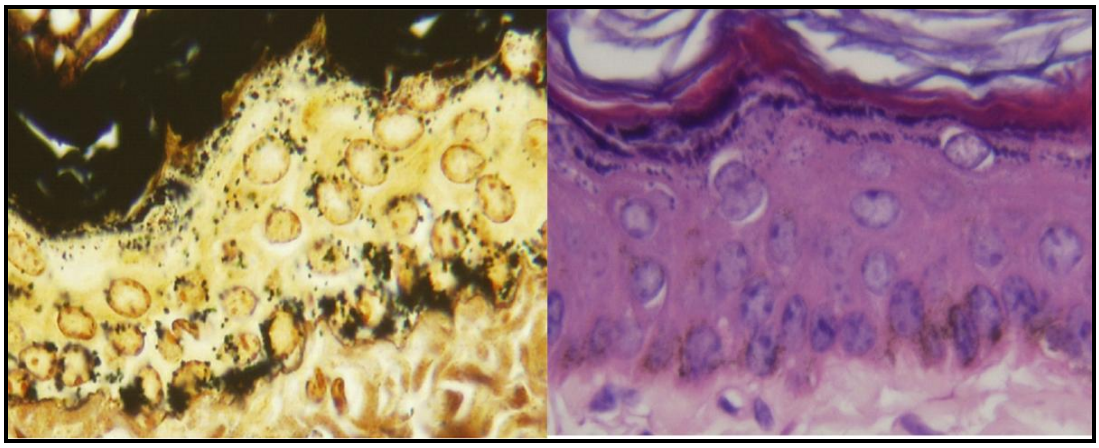

Figure: 4a) Steiner stained skin section 4b) H&E stained skin section

The preliminary data shows the average thickness of the pHGP's epidermis to be lower than the values for Yucatans and humans. The thickness for both the mini-pig and humans was 68µm while the guinea pig was about 37µm. However, there is a lot of variation in the epidermal thickness of Yucatans and humans. Areas with lower values were more in line with the numbers seen for the pHGPs. Thus, it is feasible that guinea pig skin can be substituted for the Yucatan.

Blood sample analysis showed a protein concentration of 35-41 µg/mL. This is a sufficient amount, ensuring that genomic and proteomic testing could be easily performed with HGP

blood. With the increased interest in proteomic studies, these results show that pigmented hairless guinea pigs have potential as subjects in this area.

Unfortunately, even though the pigmented hairless guinea pig shows great promise for laser skin studies, the limited availability of the animal may exclude it from use. Additionally, the unpredictable nature of this coloring makes their use in skin studies highly suspect; surface area available for exposure will be inconsistent not only between different animals, but also on the same animal. So even though skin thickness and average melanin distribution for this breed appears to make them good candidates for skin damage studies, the variable skin patterns and limited breeder availability make this animal unsuitable for experimental purposes at this time.

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